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REGULAR ARTICLE

Growth and maintenance costs of leaves and roots in two populations of *Quercus ilex* native to distinct substrates

Raquel G. Laureano · Ana García-Nogales · José I. Seco · Jesús G. P. Rodríguez · Juan C. Linares · Feliciano Martínez · José Merino

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Summary

Aims This work tests the hypothesis that growth and maintenance costs of plant organs are higher in more stressful soils.

Methods Two populations of *Quercus ilex* L were selected in the southern Iberian Peninsula, these growing in similar climates but different soil types, namely a brown well-developed soil on slate rock, and a stressful lithosol on gypsum rock. In both localities, growth and maintenance respiration were measured in undetached young and mature leaves (trees under natural conditions) and fine roots (hydroponically grown seedling).

Results Young leaves of the two populations displayed an almost identical growth cost (1.53 g glucose g^{-1}). The maintenance cost was higher in the young (40.2 vs. 25.3 mg glucose g^{-1} day^{-1}; $P<0.05$) and in the mature (7.64 vs. 4.33 mg glucose g^{-1} day^{-1}; $P<0.001$) leaves of individuals growing in gypsum soils. The growth cost of fine roots was the same in both populations (1.18 g glucose g^{-1}) while the maintenance cost was higher in the Gypsum population (8.95 vs. 7.39 mg glucose g^{-1} day^{-1}; $P<0.01$).

Conclusions The results show for first time that the cost of organ maintenance may be related to the degree of soil stress in their native habitats.

Keywords Evergreen leaves · Growth respiration · Maintenance respiration · Mediterranean species · Soil stress · Root respiration · Sclerophylly · Ecotypes

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R. G. Laureano · A. García-Nogales · J. I. Seco · J. G. P. Rodríguez · J. C. Linares · F. Martínez · J. Merino (✉)
Department of Physical, Chemical and Natural Systems, University Pablo Olavide,
Carretera de Utrera, Km 1,
41013 Seville, Spain
e-mail: jamerort@upo.es

Introduction

Habitat-related differences in growth and maintenance costs, and thus in carbon balance, may be important to explain differences in growth rates (Lambers et al. 2008; Laureano et al. 2008), plant production (Amthor 2010; Hansen et al. 2008), or species distribution (Williams et al. 1989). In the last 40 years, classical studies on habitat-respiration relationships have focused on the pattern of respiratory response to temperature by comparing individuals of the same species growing at different extremes of climatic temperature gradients, such as tundra vs. temperate, alpine vs. lowland (for example, see Mooney 1963), or individuals of the same species native to these habitats, but cultivated under common conditions (Lechowicz et al. 1980; Mariko and Koizumi 1993; Atkin et al. 2006). Most of these studies have demonstrated higher constitutive respiration rates in populations growing in, or native to, more stressful (either colder or warmer) habitats (Wright et al. 2006; Laureano et al. 2008). However, in such an approach, the main difficulties for interpretation (and the main

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sources of criticism), arise from both the high dependence of respiration rate on measurement temperature and the degree of acclimation of the plants to the temperature of the gardens where the plants were grown (see Wright et al. 2006 for a discussion).

Higher constitutive respiration rates in climate-stressed habitats appear to be related to higher enzymatic (protein) endowments, enabling fast growth rates according to a short growing season (Lechowicz et al. 1980; Körner 1989); allowing the synthesis of specific metabolites related with osmotic adjustment (Cavieres et al. 2000), of heat-shock proteins (Sun et al. 2002) or for defence against free radicals (Purvis 1997; Corcuera et al. 2005); or maintaining higher concentrations of metabolic and repair complexes and also repair rates (Semikhatova et al. 2009; Dixon et al. 2005; Tausz et al. 2007). In many cases, a clear relationship between high nitrogen concentration and a climate-stressed habitat has been demonstrated for the leaves and roots of herbaceous as well as woody species (Körner 1989; Ryan et al. 1996; Oleksyn et al. 1998). Also, higher defence endowments in the genus *Quercus* are evidenced by the reported higher stress resistance of individuals from more stressed habitats (García et al. 1998; Gratani et al. 2003; Ramírez-Valiente et al. 2009). The greater abundance of metabolic machinery in the tissues of climate-stressed individuals presumably results both in higher growth costs, due to the increased investment required for the synthesis of additional machinery, as well as in higher maintenance costs, due to the additional requirements for maintenance of that supplementary machinery in addition to the higher repair rates and molecular replacement.

Q. ilex is one of the most representative evergreen tree species in Mediterranean basin landscapes. This species shows significant among-population variation in both physiological and structural traits associated with local climate (Gratani et al. 2003; Sanchez-Villas and Retuerto 2007; García et al. 1998), suggesting ecotypic differentiation driven by local climate. In this line, Ramírez-Valiente et al. (2009) have shown that local climate can play key role in the genetic divergence among populations of *Quercus suber* (a close relative of *Q. ilex*) in the Iberian Peninsula. In addition to a great diversity of microclimate habitat types, the wide biogeographical area of this species includes a great variety of soils that developed on many distinct parent materials ranging from acidic to basic, from fertile to infertile, or from lithosols to well-developed soils; all of these results in a wide diversity of habitats differing in soil-related stress factors. The selective pressures generated by soil-type diversity may in turn lead to a wide range of populations, differing from each other in stress resistance and thus in terms of tissue growth and maintenance costs.

In comparison to other Mediterranean soils, lithosols on gypsum substrate represent a particularly stressful medium for plant life (Ruiz et al. 2003). Gypsum rocks are chemically unbalanced for their low content in phosphorus and their high content in calcium, potassium, and sulphate (which is toxic for the most of the agricultural species, Ernst 1998); all which inhibits organic-matter humification and nitrogen mineralization (Singh and Taneja 1977). Moreover, a neutral or slightly basic pH, results in low availability of metallic oligoelements and especially phosphorus (Herrero et al. 2009). From a physical standpoint, the poor structure of these soils hampers water recharge, which exacerbates plant water stress in the dry season and, in turn, can lead to hypoxia during the wet season (FAO 1990). All this explains the low growth rates registered in different Mediterranean species (Ernst 1998) including those of the genus *Quercus* growing on gypsum soils (FAO 1990).

The present study compares the growth and maintenance costs of leaf and root tissues in *Q. ilex* individuals from two areas with similar climates but contrasting soil types. In two separate experiments, we considered young (expanding) as well as mature (not expanding) leaves in adult trees growing under natural conditions, and young roots from seedlings growing in controlled (hydroponic) cultures. In a third experiment, we used seedlings grown in controlled chambers for estimating growth rates and plant traits. Growth and maintenance components were separated by gas-exchange methods following Hesketh et al. (1971) and Cannell and Thornley (2000). We postulated that both costs would be higher in individuals native to gypsum soils, since the greater stress in that soil type would lead to higher concentrations of metabolic endowments, including induced and constitutive tissue defence and repair complexes as well as higher repair rates.

Material and methods

We selected two *Q. ilex* populations in the southern Iberian Peninsula: one located on a well-developed brown, siltstone soil on slate rock, classified as a typic

Haploxeralf, (hereafter "Siltstone" population, 37°48′ N; 5°41′W) and the other on an unstructured soil (lithosol) over gypsum rock, classified as a Xerorthents, (hereafter "Gypsum" population, 37°7′ N; 5° 8′W). The two study sites were ~120 km apart. The average climate differences between the sites were small, in terms of annual rainfall (614 vs. 598 mm), annual mean temperature (16.5 vs. 17.0°C) and solar energy (2043 vs. 2036 KJ m^{-2} day^{-1} μm^{-1}) for the Gypsum and Siltstone locations, respectively. The data were gathered during the spring (the *Q. Ilex* growing season) a time of the year with frequent rainfall episodes and mild temperatures. During the sampling period, differences between the study sites were also small in terms of rainfall (122 vs. 129 mm), minimum temperature (9.7 vs. 4.8°C), and maximum temperature (21.6 vs. 20.6°C) for both locations. On the contrary, the two sites differed substantially with respect their soil characteristics. Metal (Cu, Zn, and notably Fe and Mn) concentrations and available P and N were lower in the gypsum soil, while exchangeable Ca, K and pH were higher (Table 1). Also, sites differed in stand structure (adult trees); with both stand density (21.2±6.2 vs. 33.3±3.8 individuals Ha^{-1}; $P<0.05$) and average size (diameter of the canopy) (7,3±3.1 vs. 15.4±3.4 m; $P<0.001$) being significantly lower at the Gypsum location.

Table 1 Soil characteristics of the Siltstone and Gypsum habitats

Population	Siltstone	Gypsum
Cation-Exchange Capacity (meq/100 g)	6.52	6.52
Exchangeable Calcium (meq/100 g)	2.19	Saturated
Exchangeable Magnesium (meq/100 g)	0.89	0.56
Exchangeable Potassium (meq/100 g)	0.03	0.41
Exchangeable Sodium (meq/100 g)	0.05	0.06
Available Phosphorus (μg g^{-1})	13.00	1.00
Available Potassium (μg g^{-1})	110	170
Available NO_3^- (μg cm^{-2} day^{-1})	0.65	0.18
Available NH_4^+ (μg cm^{-2} day^{-1})	0.14	0.01
Total Copper (μg g^{-1})	1.30	0.50
Total Iron (μg g^{-1})	188.20	6.50
Total Manganese (μg g^{-1})	127.20	13.80
Total Zinc (μg g^{-1})	3.00	1.30
Organic Matter (μg g^{-1})	9.20	17.00
Salinity (mmhog cm^{-1})	0.15	2.30
pH 1:2.5	6.10	7.60
C:N Ratio	8.47	8.08

Seedling morphology and growth rate

For the study, adult trees of comparable size (and presumably similar in age) were considered. Acorns gathered from 50 trees per population (roughly 20 acorns per tree) were pooled and placed in trays for germination. One month after germination, seedlings in poor condition were discarded and 50 seedlings (less than 10 cm tall) of each population were selected and placed in 2-litre pots (one per plant) using a 1:1 vermiculite-sand substrate. Average acorn dry weight (including coat) was the same in both populations (4.22±0.27 g). Twenty seedlings from each population were used to estimate the mean dry weight of the individuals of each population (initial weight). Pooled seedlings from both populations were placed in each of two growth chambers under the following conditions: 14-h photoperiod; 325 μmol m^{-2} s^{-1} PAR at leaf height; day/night temperatures 24°C/18°C; relative humidity 30–35 %. Plants were watered on alternate days with diluted (1:3) Hoagland solution to avoid nutrient deficiency and water stress. The plants were rotated both inside the chamber (roughly every 4 days) and between chambers (roughly every 10 days) to minimize the chamber effect.

The experiment lasted for 120 days. Each seedling was then divided into its stem, leaf, and root fractions, and fresh leaf surfaces measured. Fractions were oven dried at 80°C and weighed (final mass). The relative growth rate (RGR, mg g^{-1} day^{-1}) of each individual was calculated from the initial mass (the same for all the individuals of the populations) and from the final mass of the individual. The ratios LMR (mass of leaves to plant mass), LAR (total surface of leaves to plant weight), SLA (fresh leaf surface to leaf dry mass), and S:R (shoot to root mass) were also calculated for each individual. The values calculated for each individual were used to calculate the mean values for each population.

The leaf photosynthetic rate was estimated under the same conditions of temperature, relative humidity, and light intensity as those of cultivation. The determinations were made on attached individual leaves located near the middle of the stem of randomly

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selected plants at the middle of the experimental period using a gas-exchange system (CIRAS 1 PP Systems, Edinburgh, U.K.).

Leaf growth and maintenance respiration

Respiration and growth rates of attached young (developing) leaves were measured on trees growing under natural conditions. The growth rate of each leaf was estimated over a 3-day period. Leaf-surface area was measured on day 1 and day 3 making leaf images with the aid of film paper. The leaf was removed on day 3, washed, dried at 80°C, weighed, and the specific leaf area (SLA) was determined as the ratio of leaf area to leaf dry mass. SLA proved to be constant throughout the three-day period and the increase in leaf area was used to estimate the leaf-mass gain over the period. The mass increase was used to calculate the specific growth rate (SGR) as the difference in ln (mass) divided by days of growth. On day 2, respiration rate in darkens was measured (CO_2 evolution) at 20°C, with an open portable gas-exchange system based on that described by Field et al. (1982). Measurements were taken until a stable respiration rate was reached (less than 60 min). The specific respiration rate (SRR) for each leaf was calculated by dividing the respiration rate by mean leaf mass over a 3-day period. Measurements were made for a total of 43 and 32 leaves (about two leaves per tree) of the Gypsum and Siltstone population, respectively. A linear regression of SRR was performed against SGR for each population. The slope (mg CO_2 g^{-1}) represents respiration associated mainly with tissue synthesis (growth respiration), whilst the Y intercept (mg CO_2 g^{-1} day^{-1}) represents the respiration rate at zero growth, i.e. respiration associated mainly with tissue maintenance (maintenance respiration; Hesketh et al. 1971).

Maintenance respiration was also estimated in the same trees by quantifying respiration in mature (fully expanded) leaves (around 12 months old), assuming that, in the absence of growth, total respiration was related largely to maintenance processes (Cannell and Thornley 2000). The specific respiration rate of each attached leaf was estimated by dividing its respiration rate by its leaf mass. We measured 16 leaves (about one per tree) for each population, and the mean value of all measurements was taken as the leaf-maintenance respiration for the population.

Root growth and maintenance respiration

Acorns of roughly the same size were selected and placed on a surface of moist sterile sand in order to induce radicle emergence. Once the root was 6 cm long, (around 20 days later) it was transferred to a hydroponic medium in a growth chamber. The day of transfer was taken as seedling age zero. Hydroponic cultivation was carried out using 100-litre tanks containing nutrient solution, stirred with two 5 W air compressors to ensure adequate aeration and uniform growth medium. The growth medium was renewed at least once a week to avoid nutrient depletion.

Growth rates of root systems in each population were estimated using multiple harvests. Thus, every 3 days, the complete root system of a number (five-ten) of seedlings was collected. A total of eight successive collections, corresponding to eight age classes (from 1 day to 24 days old), were made. After each collection, the root fraction was separated from the aerial part of each seedling, washed, dried at 80°C for 48 h and weighed, thus providing the dry mass of the whole-seedling root system. For each age-class considered, the mean root mass of all sampled seedlings was estimated. In total, 83 and 80 root systems for the Gypsum and Siltstone populations, respectively, were considered. Linear and exponential regression models of dry root weight vs. age were established for each population. These growth equations were used as predictors to calculate the SGR of the seedlings in which SRR had been estimated.

For root respiration, the open continuous-flow system described by Martínez et al. (2002b) was used, enabling the measurement of oxygen uptake by the roots of intact seedlings. Essentially, the system consisted of an open circuit connected to a nutrient-solution container. The circuit included a chamber equipped to house the root system of a seedling 25-cm high, and an oxygen electrode (Hansatech Ltd, United Kingdom) to measure the concentration of dissolved oxygen in the chamber solution. During the experiments, the root chamber was kept in darkness at 20°C, whereas the above-ground portion of the seedling was kept light at 400 μmol m^{-2} s^{-1} PAR at a constant temperature of roughly 23°C; these conditions were very close to the growing conditions. Respiration was measured at ages ranging from 2 to 24 days, in 21 and 27 seedlings for each population. After each respiration measurement, roots were separated, washed,

dried at 80°C, and weighed, and SRR for each age class calculated.

Mass-age regressions in root systems proved to fit ($P<0.001$) the linear model in both populations (not shown). On the basis of these linear equations, and for each population, the SGRs (mg g^{-1} day^{-1}) of root systems were calculated for each age interval for which the specific respiration rate (SRR) was estimated; and the linear regression of SRR on SGR was performed for each population.

Growth and maintenance costs

The growth cost was estimated as the sum of the substrate used in growth respiration (6 Mol CO_2 equals 1 Mol glucose) plus the carbon stored in the form of tissue-mass increase (6 Mol C equals 1 Mol glucose) during organ growth (Hesketh et al. 1971). Values were expressed in glucose equivalents (g glucose g^{-1} dry mass). The maintenance cost was derived directly from maintenance respiration and was expressed in mg glucose g^{-1} dry mass day^{-1}.

Respiration response to temperature and carbon and nitrogen concentration

To ascertain the effect of temperature measurement on respiration, a leaf of intermediate age (i.e. between young and mature) was selected from each of trees totalling 4–5 leaves for each population. The respiration rate for each attached leaf was measured at five temperatures (from 10 to 30°C) following the procedure described above, but changing the cuvette air temperature following an aleatory sequence. The experiment was repeated for the undetached whole root systems of eight hydroponically grown seedlings (four from each of the two populations) over the temperature range 7–25°C. For each population, the linear and exponential regressions of organ respiration vs. temperature were established.

The leaves (young and mature) and root systems considered in the respiration analysis were ground individually. The N and total C concentrations of each organ were then measured using an elemental analyser (LECO Corporation, St. Joseph, Michigan, USA) and the results were expressed as mg nitrogen g^{-1}, and carbon as a percentage of mass. A total of 38 and 39 leaves plus 16 and 16 root systems were considered for Gypsum and Siltstone populations, respectively. To test the effect of foliar N on respiration in both populations the ratio SRR:N was calculated and then average population values were compared by ANOVA (see below), using leaf age as an additional explanatory variable.

Statistical analysis

Root mass was regressed against age using two models (linear and exponential) in order to estimate root-growth equations for each population. For the estimation of growth and maintenance respiration (Rg and Rm, respectively), SRR was regressed (linear model) against SGR. For the detection of differences in Rg and Rm values between populations, regression lines for SRR vs. SGR were subjected to a parallelism test and to Tukey's test (Zar 1999). The SRR:N ratios were compared by Factorial ANOVA, using soil type (gypsum vs. siltstone) and leaf age (young vs. mature) as explaining variables; *post hoc* comparisons were performed using Tukey's test; and the significance level was fixed at $P<0.05$ in all cases. Statistical analyses were performed using the software STATISTICA (Stat Soft, Inc. –2005–. Tulsa, Oklahoma, USA).

Results

Seedling-biomass allocation and growth rate

Under controlled growth conditions, the seedlings of the two populations showed similarities in biomass allocation (S:R), in LMR, and in the photosynthetic rate (Table 2); however, the seedlings of the Gypsum population had a greater SLA ($P<0.05$), resulting in a greater LAR ($P<0.05$) than in the Siltstone population. Nevertheless, the Gypsum seedlings displayed a lower growth rate (RGR) than did the Siltstone ones (19.4 vs. 26.0 mg g^{-1} day^{-1}; $P<0.01$).

Growth and respiration in young and mature leaves

In adult trees, the young leaves displayed the same C and N concentration averages in both populations (Table 3), but differed in a set of structural and physiological traits. Thus, Gypsum trees had smaller leaf-blade size (LS) ($P<0.001$) and SLA ($P<0.001$) but higher leaf SRR ($P<0.001$). Mature leaves showed a pattern similar to that in young leaves (Table 4).

Table 2 Means (± SD) of variables measured in *Q. ilex* seedlings from Gypsum and Siltstone populations cultivated in growth chambers (see text for explanation). Asterisks denote significant differences between populations (*: $P<0.05$; **: $P<0.01$;). Abbreviations: n = number of seedlings considered; LS = leaf-blade size; LMR = leaf-mass ratio; LAR = leaf-area ratio; S:R = shoot to root ratio; SLA = specific leaf area; RGR = relative growth rate; and A = photosynthetic rate

Population	Siltstone	Gypsum	
n	32	43	
LS (cm^2)	2.82±0.73	2.34±0.66	n.s.
LMR	0.31±0.04	0.31±0.06	n.s.
LAR ($m^2\ kg^{-1}$)	1.60±0.15	1.96±0.39	*
S:R	0.77±0.15	0.81±0.70	n.s.
SLA ($m^2\ kg^{-1}$)	5.44±0.52	6.41±0.84	*
RGR (mg g^{-1} day^{-1})	26.0±2.2	19.4±3.6	**
A (mg CO_2 m^{-2} s^{-1})	0.54±0.18	0.53±0.17	n.s.

The specific respiration rate correlated positively ($P<0.01$) with the specific growth rate of young leaves for both populations (Fig. 1). There was no significant difference in slope (growth respiration, Rg) between the two populations; thus, growth respiration per unit mass in young leaves (Rg, Table 3) was the same in the two populations studied (average: 0.56±0.12 g CO_2 g^{-1}, equivalent to 0.38 g glucose g^{-1}), as was the C concentration (average 461±5 mg g^{-1}, equivalent to 1.15 g glucose g^{-1}). Therefore, the average growth cost (G_{cost}) (the sum of the growth respiration cost plus carbon-skeleton cost) of the study populations was similar, at 1.53±0.1 g glucose g^{-1}. Intercept (maintenance respiration, Rm) was higher ($P<0.05$) in the Gypsum population than in the Siltstone one (59.1±8.1 vs. 37.2±6.7 mg CO_2 g^{-1} day^{-1}, Table 3) and therefore so was the maintenance cost (40.2±5.5 vs. 25.3±4.6 mg glucose g^{-1} day^{-1}, $P<0.05$).

In mature leaves (Table 4), specific respiration rate was higher ($P<0.001$) in the Gypsum population (10.56±2.07 vs. 6.37±1.99 mg CO_2 g^{-1} day^{-1}), this being equivalent to a maintenance cost of 7.64±1.41 mg glucose g^{-1} day^{-1} and 4.33±1.35 mg glucose g^{-1} day^{-1} ($P<0.001$) for the Gypsum and Siltstone populations respectively.

Table 3 Means (± SD) of variables measured in young leaves of *Q. ilex* adult trees growing under field conditions from the two study populations, and regression coefficients for specific respiration rate (SRR) vs. specific growth rate (SGR) in the same leaves. Asterisks denote significant differences between populations (*: $P<0.05$; ***: $P<0.001$). Abbreviations: n = number of sampled leaves; LS = leaf-blade size; SLA = specific leaf area; [C] = carbon concentration; [N] = nitrogen concentration; Rg = growth respiration; Rm = maintenance respiration; G cost = growth cost; M cost = maintenance cost

Population	Siltstone	Gypsum	
n	32	43	
LS (cm^2)	3.16±0.94	1.64±0.69	***
SLA ($m^2\ kg^{-1}$)	10.61±1.99	7.55±0.80	***
[C] (mg g^{-1})	462±6	460±4	n.s.
[N] (mg g^{-1})	18.3±2.0	17.4±1.1	n.s.
SGR (mg g^{-1} day^{-1})	63.8±37.7	53.7±25.2	n.s.
SRR (mg CO_2 g^{-1} day^{-1})	66.8±25.6	94.3±25.3	***
Rg (g CO_2 g^{-1})	0.46±0.09	0.65±0.14	n.s.
Rm (mg CO_2 g^{-1} day^{-1})	37.2±6.7	59.1±8.1	*
G cost (g glu g^{-1})	1.47±0.08	1.60±0.11	n.s.
M cost (mg glu g^{-1} day^{-1})	25.3±4.6	40.2±5.5	*

Table 4 Mean values (± SD) of variables measured in mature leaves of *Q. ilex* adult trees growing under field conditions from the two study populations. Asterisks denote significant differences between populations (***: $P<0.001$). Abbreviations: n = number of sampled leaves; LS = leaf-blade size; SLA = specific leaf area; [N] = nitrogen concentration; A = photosynthetic rate ($n=30$ for both populations); SRR = specific respiration rate; M cost = maintenance cost

Population	Siltstone	Gypsum	
n	16	16	
LS (cm^2)	7.06±2.15	3.71±1.68	***
SLA ($m^2\ kg^{-1}$)	5.15±0.05	3.45±0.03	***
[N] (mg g^{-1})	12.5±0.8	13.6±0.5	n.s.
A (mg CO_2 m^{-2} s^{-1})	0.37±0.14	0.37±0.15	n.s.
SRR (mg CO_2 g^{-1} day^{-1})	6.37±1.99	10.56±2.07	***
M cost (mg glu g^{-1} day^{-1})	4.33±1.35	7.64±1.41	***

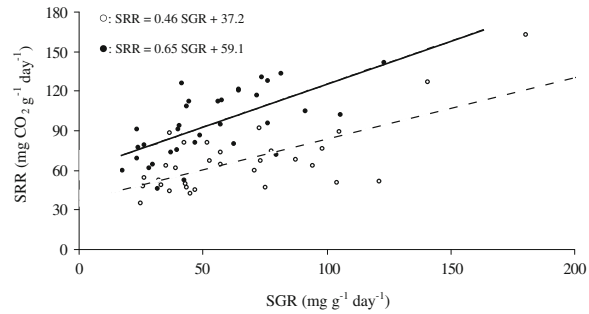

Fig. 1 Specific respiration rate (SRR) vs. specific growth rate (SGR) in young leaves of Gypsum and Siltstone populations of *Q. ilex* (field conditions). Gypsum population: solid line and solid symbols ($r=0.65$; $P<0.001$). Siltstone population: broken line and blank symbols ($r=0.68$; $P<0.001$)

t5.1 **Table 5** Mean values (± SD) of variables measured in young root systems of *Q. ilex* seedlings growing in hydroponic cultures, and regression coefficients for specific respiration rate (SRR) vs. specific growth rate (SGR) in the same root systems (all the values in dry weight). Asterisks denote significant differences between populations (**: $P<0.01$). Abbreviations: n = number of sampled root systems; [C] = carbon concentration ($n=10$ for both populations); [N] = nitrogen concentration ($n=10$ for both populations); Rg = growth respiration; Rm = maintenance respiration; G cost = growth cost; M cost = maintenance cost

t5.2	Population	Siltstone	Gypsum	
t5.3	n	27	21	
t5.4	[C] (mg g^{-1})	430±5	418±8	n.s.
t5.5	[N] (mg g^{-1})	23.3±4.1	23.8±2.8	n.s.
t5.6	SGR (mg g^{-1} day^{-1})	121.9±70.4	98.6±47.0	n.s.
t5.7	SRR (mg O_2 g^{-1} day^{-1})	21.8±9.6	24.0±7.4	n.s.
t5.8	Rg (g O_2 g^{-1})	0.11±0.01	0.15±0.01	n.s.
t5.9	Rm (mg O_2 g^{-1} day^{-1})	7.88±2.09	9.54±1.56	**
t5.10	G cost (g glu g^{-1})	1.18±0.03	1.18±0.03	n.s.
t5.11	M cost (mg glu g^{-1} day^{-1})	7.39±1.96	8.95±1.45	**

Growth and respiration in the root system

The young roots of the seedlings of the two populations displayed the same C and N concentrations (Table 5). Also, no significant differences were found either in the mean SGR or in the mean SRR of the root systems of the two populations considered.

In both populations, the regression line of SRR on SGR was significant ($P<0.01$; Fig. 2). There was no significant difference in slope (Rg, Table 5) between the two populations (average 0.13±0.02 g O_2 g^{-1}, equivalent to 0.12 g glucose g^{-1}), as was the case with the C concentration (average 424±7 mg g^{-1}, equivalent to1.06±0.02 g glucose g^{-1}). Therefore, the average growth cost (G_{cost}) for the two populations was 1.18±0.03 g glucose g^{-1}.

The intercept of the regression lines (maintenance respiration, Rm) was higher in the Gypsum population (Table 5) than in the Siltstone one (9.54±1.56 vs. 7.88 ±2.09 mg O_2 g^{-1} day^{-1}; $P<0.01$), and therefore so was the maintenance cost (8.95±1.45 vs. 7.39±1.96 mg glucose g^{-1} day^{-1}, $P<0.01$).

Effect of tissue N concentration and temperature on respiration

A linear relationship ($P<0.01$) was noted between SRR and N concentration. The SRR:N ratio was significantly related to soil type and leaf age, with the full model explaining a 78 % of the total observed variance ($P<0.001$). The soil type showed a significant effect regardless of the leaf age ($P<0.001$; 4 % of variance explained), indicating that the respiration rate per unit of foliar N concentration was on average higher in the gypsum than in siltstone soil (Fig. 3). Leaf age was the most significant effect ($P<0.0001$; 72 % of variance explained), showing that the respiration rate per unit of foliar N concentration was higher in young leaves than in mature ones (Fig. 3). The soil type x leaf age interaction was also significant ($P=0.02$; 2 % of variance explained), indicating that the age effect on the respiration rate per unit of foliar N concentration was higher in gypsum than in siltstone soil.

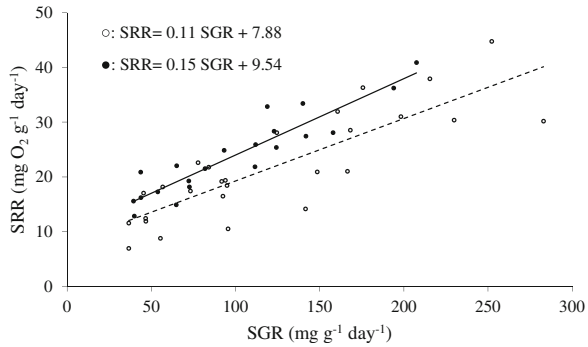

Fig. 2 Specific respiration rate (SRR) vs. specific growth rate (SGR) in young roots of Gypsum and Siltstone populations of *Q. ilex* (hydroponic conditions). Gypsum population: solid line and solid symbols ($r=0.92$; $P<0.01$). Siltstone population: broken line and blank symbols ($r=0.84$; $P<0.01$)

Leaf-respiration response to temperature was linear in both populations (Fig. 4). Regression lines displayed similar slopes in both populations, but intercepts were higher in the leaves (Fig. 4a) and roots (Fig. 4b) of the Gypsum population; indicating that Gypsum population organs had higher ($P<0.01$) respiration rates than did the Siltstone ones at the same temperature.

Discussion

Both, the strong chemical imbalance (i.e. excess of calcium and probably sulphate) and the low availability of some critical nutrients (P, N, Fe or Mn) in gypsum soils (Table 1) are associated with both a smaller tree size and lower stand density; suggesting that gypsum soils pose significant problems for *Q. ilex* performance.

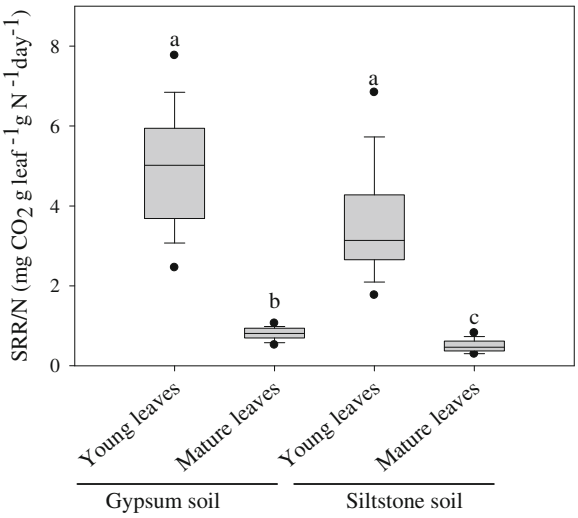

Fig. 3 Leaf specific respiration rate (SRR) per unit of foliar nitrogen concentration (N) in different soil types (gypsum and siltstone) and leaf ages (young and mature) of *Q. ilex* trees growing under natural conditions. In the box-plot figure, error bars represent the 5th/95th percentiles; boxes represent the standard errors (n=16); solid lines represent the means and points are outliers. Different letters represent significant differences for Factorial ANOVA, P<0.05

Plant structure and growth

Species growing along environmental gradients show interspecific relationships among plant traits such as those related to leaf structure and function, and plant growth. Thus, low resources (nutrients, water) or otherwise stressed habitats appear to select for lower leaf-blade size and specific leaf area, lower photosynthetic and respiration rates, and long-lived and more conservative leaves (i.e. higher nutrient and water-use efficiency, higher defence endowments), all of which result in lower plant-growth rates, (Reich et al. 1997; Cavender-Bares et al. 2004). In the present study, the significance of differences in leaf traits between the two populations of the same species are presumably limited because of intraspecific genetic constraints (Cavender-Bares et al. 2004); however, relationships in the traits of adult trees reflect the expected trends for individuals growing along stress gradients: comparatively smaller (lower LS) and thicker (lower SLA) leaves in trees growing in the more stressed (Gypsum) habitat (Tables 3 and 4). It bears noting that the specific respiration rate was higher in the leaves of the Gypsum population as was the respiration rate per unit of nitrogen (Fig. 3), contrary to what was expected, since stress conditions are usually associated to less active organs (Lambers et al. 2008).

Growth conditions may alter the population rank of plant traits. Thus, as opposed to trees growing under the natural conditions discussed above, Gypsum seedlings growing in controlled non-limited cultures displayed a higher SLA (which resulted in a higher LAR; Table 2). Despite these differences and the fact that both the average photosynthetic rate (A) and plant allocation (S:R) were the same for both populations, the seedlings of the Gypsum population displayed a

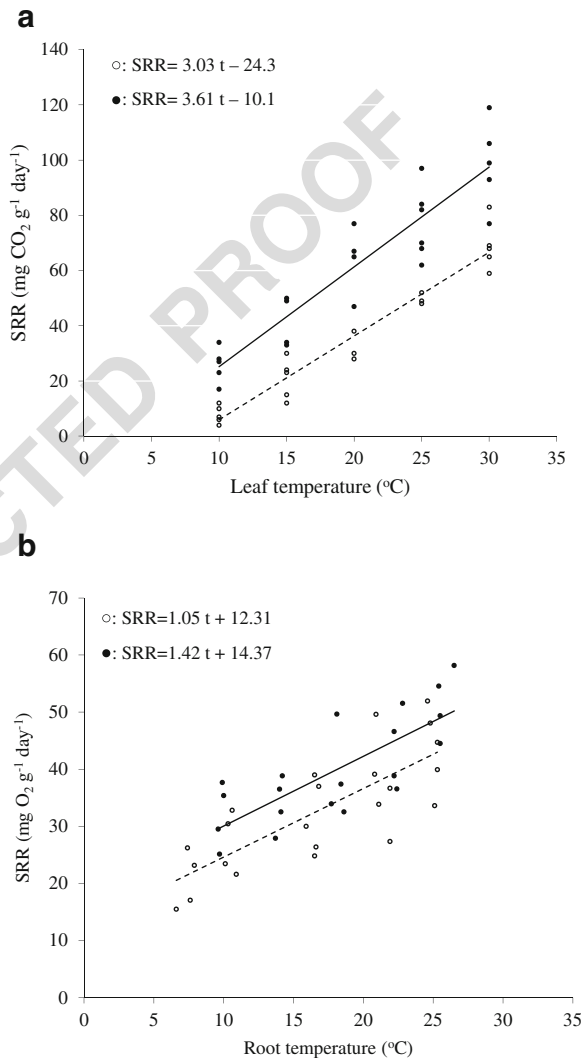

Fig. 4 (**a**) Response of leaf specific respiration rate (SRR) to temperature in Gypsum and Siltstone populations of *Q. ilex* (field conditions). Gypsum population: solid line and solid symbols (r=0.92; P<0.001), Siltstone population: broken line and blank symbols (r=0.96; P<0.001). (**b**) Response of root specific respiration rate (SRR) to temperature (hydroponic conditions). Gypsum population (r=0.78; P<0.01), Siltstone population (r=0.79; P<0.01)

lower RGR, which is opposite of what was expected, since higher SLA values are usually associated with higher growth rates (Lambers et al. 2008; Wright and Westoby 1999).

All together, these results suggest that in the Gypsum population, organ respiration rates were higher than expected with regard both to the leaf traits and to the relative growth rate of the individuals.

Growth cost

The results do not support the hypothesis that organ-growth costs were higher in the more stressed (Gypsum) population than in the less stressed (Siltstone) one. The mean growth cost (1.53±0.10 g glucose g^{-1}) for young *Q. ilex* leaves in the present study was almost the same as that reported by Villar and Merino (2001) for mature *Q. ilex* leaves growing under natural conditions, but higher than that published by Laureano et al. (2008) for young leaves of this species growing in hydroponic medium. The mean root-growth cost (1.18±0.03 g glucose g^{-1}) was lower than reported by Martínez et al. (2002a) for young roots of seven *Quercus* species growing under natural conditions in SW Spain, but comparable to the values of the same seven species growing in hydroponic cultures (Martínez et al. 2002b); and almost identical to those published by Laureano et al. (2008) for two populations of *Q. ilex* grown under hydroponic conditions. Root systems are notable importers of organic molecules synthesised by aboveground organs, which are subsequently used in root growth. Therefore, a portion of the root-respiration expenditure (associated with root growth) is not computed as root-growth cost. Besides, a significant portion of the root-growth respiration is associated with the uptake of nutrients required for root growth. Since the nutrient concentration in the medium solution was comparatively high (hydroponics), the energy cost of nutrient uptake was probably low; all the above explains the low root-growth cost found in the present study (Table 5). Along the same line, a major fraction of root-maintenance respiration is associated with the maintenance of ion concentrations in the internal root medium. The small ion gradients, because of the high nutrient concentration in the hydroponic medium, would require a minimal energy expenditure, which would result in the low root-maintenance respiration values recorded (Table 5).

Also, in non-limited environments (e.g. hydroponic cultures), selection tends to favour tissues proportionally both richer in cellulose (a low-cost component) and lower in wax content (a costly component) (Martínez et al. 2002a). The favourable (hydroponic) growth conditions of seedlings from which root systems were analysed in the present study (no water or nutrient limitation) may account for the low growth costs observed for roots as compared with those of plants growing in natural conditions. This may explain also the higher growth cost of the leaves of plants growing under natural conditions (present study) as compared with published values for *Q. ilex* leaves growing in hydroponic cultures (Laureano et al. 2008).

The absence of a significant difference in growth cost between the two compared populations agrees with the absence of differences in tissue thiol concentrations (an indicator of the abundance of defensive endowments) in the leaves and roots of both populations (Laureano and de Kock, unpublished). The constant growth cost of a given organ in a given species has been explained as a result of its constant chemical composition (Penning de Vries et al. 1974), the correlations among different chemical fractions of their constituent tissues (Martínez et al. 2002a), or the existence of genetic limits for organ growth cost (Merino 1987). Our results suggest that growth-cost values are rather independent of environmental factors (i.e., siltstone vs. gypsum soils). However, results also show that significant differences in growth cost can be found when strongly contrasting growth conditions are compared, such as those of natural conditions compared to hydroponic cultures.

Maintenance cost

The results support the hypothesis that stressed populations expend more energy on maintenance than do less stressed ones. It bears noting that despite considerable differences due to either organ type (leaves vs. roots), age (young vs. mature leaves), or growth conditions (hydroponic cultures [roots] vs. natural conditions [leaves]), maintenance costs in the Gypsum population were significantly greater than in the Siltstone one (Tables 3, 4, and 5), suggesting that the results were robust. This pattern fits very well with the results of a previous study concerning two *Q. ilex* populations native to two distinct climatic areas, cultivated under homogeneous hydroponic conditions (Laureano et al. 2008). Maintenance costs of the root systems in the present study (hydroponic cultures)

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were almost identical to those reported by Laureano et al. (2008), while maintenance costs of the leaves in the present study (natural conditions) were higher, as expected, since natural conditions should be more stressful than those of hydroponic cultures.

The observed trends in maintenance-respiration differences between populations were not an artefact of temperature. Thus, Gypsum and Siltstone trees were growing at roughly the same temperature as were the seedling native to these habitats growing in controlled cultures. Thus, neither the habitat temperature nor cultivation temperature could be responsible for the higher maintenance respiration detected in the organs of plants native to the more stressful habitat (Gypsum). Also, in all the cases, respiration rates were measured at growth temperature; therefore, the effect of measurement temperature should be excluded. Finally, it is important to underline that the response pattern of respiration to measurement temperature, either in root systems or in leaves, was similar in both populations (Fig. 4), indicating that observed differences between populations in maintenance respiration were not a consequence of the measurement (20°C) temperature (Tjoelker et al. 1999; Atkin et al. 2006; Zaragoza-Castells et al. 2007). Besides, no differences were found either in annual or in seasonal (spring) rainfall between the two localities considered. Thus, presumably, comparable rainfall between sites resulted in adequate and similar soil-water availability even though the soil texture differed between sites. According to all the above, we conclude that the interpopulation differences in organ respiration rate could be related to between-habitat differences other than climatic ones. Thus, the results confirm for the first time that soil stress (and not only climatic stress) may induce higher maintenance costs.

In gypsum soils, the combination of the serious nutritional limitations of Fe, Mn, N and P, and the excess of calcium (and possibly sulphate) in the tissues, would demand strong enzymatic endowments, both for nutrient uptake, transport, vacuole storage, or secretion (Lambers et al. 2008). Besides, the low availability of P and/or the hypoxia periods can influence the continuity of the electron flow in the respiratory chain, such that the water limitation can block the dark phase of photosynthesis, with the result of free-radical formation in mitochondria and chloroplasts (Sun et al. 2005), while the excess of Ca cations can result in free radical formation in root cells (Minibayeva et al. 2000). All the above would require a comparatively larger energy investment in the synthesis and maintenance of defence and repair systems (Purvis 1997), thus explaining the higher rate of plant respiration observed in gypsum soils (Rakhmankulova et al. 2001) as well as the greater maintenance costs noted in the present study.

Protein maintenance is the major component of maintenance cost (Bouma et al. 1994), which could account for a significant part of the correlation between leaf N concentration and respiration rate found in the present study (not shown) and already noted for several *Quercus* species (Martínez et al. 2002b; Xu and Griffin 2006), including *Q. ilex*, (Laureano et al. 2008). However, the nitrogen-respiration relationships are not straightforward. Thus, in all the cases cited above the x-intercepts of respiration vs. N concentration regression lines suggest that around 30 % of the leaf N concentration makes no contribution to leaf respiration. Also, the respiration rate per unit of N changes with leaf age, and is higher ($P<0.05$) in Gypsum leaves (Fig. 3), all together suggesting the existence of different N fractions differing from each other in their degree of activity (Vose and Ryan 2002; Wright et al. 2006) and, consequently, in their contribution to maintenance respiration.

The existence of different N fractions (i.e. reserve, structural, enzymatic) and the changes in their relative proportions with both age (Niinemets et al. 2007) and growth conditions (Ögren 2000), would weaken the total-N concentration and respiration-rate relationships, thereby explaining the lack of a significant correlation between average N-concentration difference between populations and maintenance-respiration difference. It bears mentioning that in comparisons of leaves having similar characteristics (SLA), N concentrations proved consistently higher ($P<0.01$) in the leaves of the Gypsum population (Laureano, unpublished), suggesting higher metabolic machinery and, perhaps, higher defence endowments (see Laureano et al. 2008 for a discussion). In addition, differences in maintenance respiration between populations observed in the present study might be related –at least in part– to higher alternative respiration-pathway activity associated with stressful soils (Martínez et al. 2003; Martinez unpublished), as has been demonstrated for stressful conditions (Florez-Sarasa et al. 2007).

In conclusion, more abundant physiological machinery in gypsum soils as indicated by higher N concentrations per unit SLA (Laureano unpublished), more active N fractions as suggested by

higher respiration rates per unit of N, and perhaps higher alternative respiration-pathway activity, would result in higher maintenance respiration in Gypsum seedlings (Tables 3, 4 and 5). This could be responsible for their lower growth rate despite both their greater SLA and LAR (Table 2), and despite of the absence of differences between populations in both photosynthetic rate and plant allocation (S:R). A similar association between stressful habitats, lower plant-growth rates, and higher maintenance costs has been reported by Laureano et al. (2008) for seedlings of *Q. ilex* native to highly contrasting climatic habitats cultivated under homogeneous conditions, suggesting that these relationships are constitutive.

Whatever the determinants of the observed maintenance-cost differences might be between populations, these appear to have important implications for the species management and conservation. Thus, in a changing environment such as that resulting from Global Change, and because of their putative higher stress resistance (García et al. 1998), populations native to stressful habitats appear to play an important role as refuges and centres for re-colonization of new empty areas. In the same line, these populations would be the most suitable for species conservation (Channell and Lomolino 2000) and restoration (Lawton 1993). However, the role of the these populations as colonisers of new empty areas is not straightforward since their constitutive higher energy requirements, lower growth rates, and presumably poor seed production (García et al. 2000) could limit the suitability of these populations to leading colonization.

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